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Micro Algae – A Review on Its Commercial Potential

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ABSTRACT:
Algae have been used as food and as sources of a wide range of chemicals for thousands of years. Over the last 50 years, the major products from algae have been the phycocolloids, namely agar and carrageenans; many macroalgal species, as well as being used as human food, have been an important source of folk medicines and are now being examined as potential sources of new drugs. Details are given of the algal products currently being commercialized, which include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, and biologically active molecules for use in human and animal health. Specific requirements for research and development for particular products are outlined. It is concluded that despite such limitations, the use of microalgae as sources of valuable chemicals is established and the next few years should see a continued expansion of the range of commercially available microalgal products.

Keywords: Biomass, Microalgae, Carotenoids, Phycobiliproteins, Polyunsaturated Fatty Acids

INTRODUCTION

Microalgal use by indigenous populations has occurred for centuries. Indeed, edible blue-green algae including Nostoc, Arthrospira (Spirulina) and Aphanizomenon species have been used for food for thousands of years [1]. However, the cultivation of microalgae is only a few decades old [2]. In the early 1950's, the increase in the world's population and predictions of an insufficient protein supply led to a search for new alternative and unconventional protein sources. Algal biomass appeared at that time as a good candidate for this purpose [3, 4]. Meanwhile, the systematic examination of algae for biologically active substances, particularly antibiotics, began [5]. Interest in applied algal culture continued with studies of the use of algae as photosynthetic gas exchangers for space travel [2]. In the USA, environmental technologies aimed at the improvement in the quality of wastewater and the fermentation of the resulting biomass to methane were implemented [6]. This use of microalgae for generating renewable energy sources provoked heightened interest during the energy crisis in the 1970's [4, 6, 7]. Commercial large-scale culture started in the early 1960's in Japan with the culture of Chlorella by Nihon Chlorella (Taipei, Taiwan) [2, 8, 9]. It was followed in the early 1970's by the establishment of an Arthrospira harvesting and culturing facility in Lake Texcoco by Sosa Texcoco S.A. (Mexico City, Mexico) [2, 8]. The first aquaculture fields also appeared in the 1970's [6]. By 1980, there were 46 large-scale factories in Asia producing more than 1000 kg of microalgae (mainly Chlorella) per month. The commercial production of Dunaliella salina, as a source of β-carotene, became the third major microalgal industry when production facilities were established by Western Biotechnology (Hutt Lagoon, Australia) and Betatene (Whyalla, Australia) (now Cognis Nutrition and Health) in 1986. These were soon followed by other commercial plants in Israel and the USA. The same as that of these algae, the large-scale production of cyanobacteria (blue-green algae) began in India at about the same time. More recently, several plants producing Haematococcus pluvialis as a source of astaxanthin have been established in the USA and India. Thus, in a short period of about 30 years, the microalgal biotechnology industry has grown and diversified significantly.

Nowadays, the microalgal biomass market produces about 5000 tones of dry matter/year and generates a turnover of approximately US$ 1.25×109/year [10]. The aim of this study is to summarize the commercial applications of microalgae. As history has shown, research studies on microalgae have been numerous and varied but they have not always resulted in commercial applications. Although recent reviews on microalgal applications exist, they generally mix actual applications and future potential

WHAT ARE MICROALGAE?

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta) [14, 15]. A more in depth description of microalgae is presented by Richmond [16]. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed [16]. During the past decades extensive collections of microalgae have been created by researchers in different countries. An example is the freshwater microalgae collection of University of Coimbra (Portugal) considered one of the world's largest, having more than 4000 strains and 1000 species. This collection attests to the large variety of different microalgae available to be selected for use in a broad diversity of applications, such as value added products for pharmaceutical purposes, food crops for human consumption and as energy source. A bit all over the world, other algae collections attest for the interest that algae have risen, for many different production purposes. For example, the collection of the Goettingen University, Germany (SAG), that started in the early 1920s and has about 2213 strains and 1273 species. About 77% of all the strains in the SAG collection are green algae and about 8% cyanobacteria (61 genera and 230 strains). Some of them are freshwater red algae and others from saline environments. The University of Texas Algal Culture Collection is another very well known collection of algae cultures that was founded in 1953. It includes 2300 different strains of freshwater algae (edaphic green algae and cyanobacteria), but includes representatives of most major algal taxa, including many marine macrophytic green and red algae species. In the Asian continent, the National Institute for Environmental Studies Collection (NIES), in Ibaraki, Japan, holds a collection of

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about 2150 strains, with around 700 species of different algae. The CSIRO Collection of Living Microalgae (CCLM), in Australia, holds about 800 strains of different algae, including representatives from the majority of classes of marine and some freshwater microalgae, being the majority of the strains isolated from Australian waters.

MICROALGAL BIOTECHNOLOGY

Biotechnology is an inter and multidisciplinary area of science, which means it is essential that there is a collaboration of effectively integrated professionals working in different fields of knowledge, such as biochemistry, physiology, genetics, microbiology, virology, botany, zoology, ecology and engineering. Biotechnology is the body of knowledge, techniques and methods, with scientific or practical basis, that allows the use of microorganisms as an integral and active part of industrial production of goods and services. Biotechnology products range from modified foods and beverages, to other industrial products such as solvents, organic acids, esters, amino acids, polysaccharides, enzymes, vitamins, antibiotics, hormones and biofuels. The use of microorganisms and their metabolic products by humans is one of the most significant fields of biotechnology activities. The knowledge of the activity of microorganisms in the conversion of certain substances into others is of great importance, as is the possibility of using a wide variety of substrates for viable products and subproducts, which enables a rational and balanced use. In Mexico, the ancient Aztecs collected algae of the genus Spirulina from alkaline lakes for food consumption. The commercial cultivation of microalgae started many years ago, when the main species were Chlorella, Spirulina, used as a food, Dunaliella salina for the extraction of b-carotene, Haematococcus pluvialis for the extraction of astaxanthin and various other species used as feed in aquaculture. Microalgae represent the prokaryotic and eukaryotic (true algae) photosynthetic microorganisms that live in an aquatic environment. Over the past 30 years, microalgal biotechnology has developed and diversified significantly. [16, 17, 18]

The cultivation of microalgae is being applied in the production of pharmaceuticals, biochemicals, fertilizers, health food, animal feed and more recently has been proposed as a source of biofuels (Fig. 1) [18]. Microalgae can be combined to produce methane (biogas), ethanol extraction, to produce hydrogen photosynthetically, and some of them accumulate lipids from which fatty acids can be extracted giving rise to biodiesel. The commercial production of Spirulina is based on raceway type open tanks, although some companies use a closed tubular bioreactor. [19, 20, 21, 22]

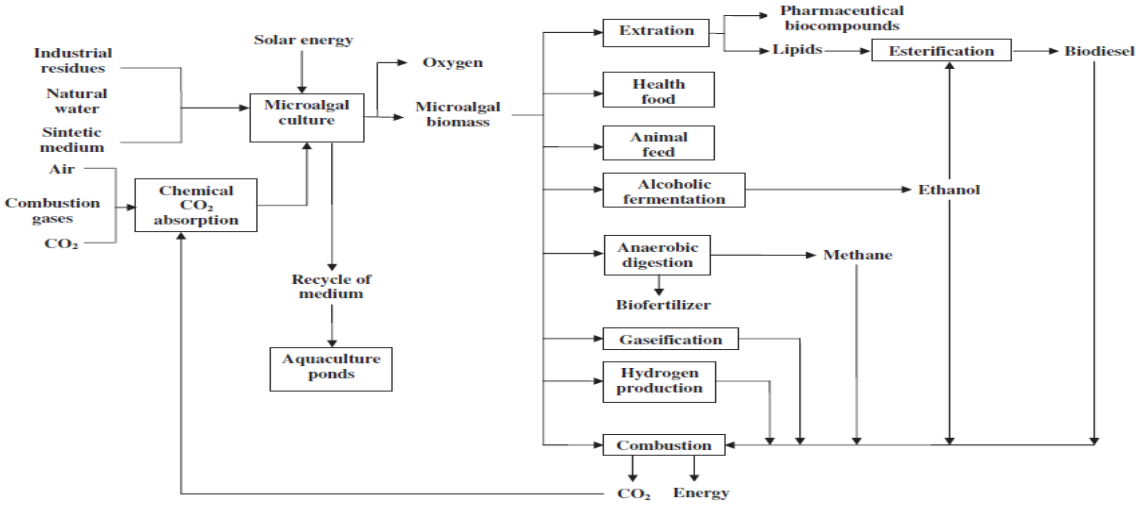

Fig. 1. Flow diagram of biomass microalgae potentialities.

Cultivation in raceway-type tanks are made in Israel, the United States and China. Circular tanks are used in Japan, Taiwan and Indonesia. In China, one company has an annual production of 200 tons of algae, which corresponds to 25% of national production and 10% of world production. The largest plant for microalgae cultivation in the world is located in Calipatria (CA, USA) in an area that is 440,000 m^2. Many companies sell nutraceuticals developed with microalgae. In Yangon (Myanmar), tablets, chips, creams and liquid extract of Spirulina are sold. In Kona (Hawaii, USA), Spirulina powder is marketed. In India, Inner Mongolia (China), Tianjin (China), Mexico, Cuba, Iran, Taiwan and Japan, industries produce Dunaliella microalgae to obtain b-carotene. The largest producers of Haematococcus on a commercial scale in the world are located in Kailuai-Kona (USA) and Chennai (India). In Brazil, the Laboratory of Biochemical Engineering (LEB), at the Federal University of Rio Grande (FURG) develops technologies for the cultivation of microalgae. Among the technologies studied and under study, the following stand out: discontinuous and semi-continuous cultivation, cultivation in open and closed photobioreactors, the effect of factors such as temperature, illuminance, aeration, replacement rate, cut-off concentration, the profile of fatty acids in microalgae, the production of biofuels from microalgae, sources of alternative nutrients, such as Mangueira Lagoon water and CO_2, production costs, simultaneous cultivation of Spirulina with toxigenic microalgae, the hypocholesterolemic potential of Spirulina platensis, the isolation of a native strain of Spirulina, the isolation of microalgae with potential for CO_2 biofixation, the mathematical modeling of the growth of Spirulina and development of nanofibers using microalgal biomass. [23, 24] Since 1998, the LEB has been developing a project studying the cultivation of Spirulina on a pilot scale on the edge of Mangueira Lagoon, for addition to meals for local children. Products that are easy to prepare, conserve and distribute have been developed. These products include: instant noodles, flan, powdered mixture for cakes, cookies,

chocolate powder, instant soup, isotonic sports drinks, gelatine powder and cereal bars. The LEB, along with the President Medici Power Plant (UTPM), operated by the Society of Thermal Electricity Generation (CGTEE) since January 2005, has carried out the cultivation of microalgae for the biofixation of CO_2 that is emitted in the combustion of coal at UTPM. Due to this project, the partners joined the International Network for Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae. With the problems that world is now facing in terms of global warming due to burning of fossil fuels and depleting resources there is a serious renaissance of interest in renewable energy from biological sources. [25, 26, 27, 28, 29].

MAJOR CLASSES AND GENERA OF CULTURED ALGAL SPECIES

Today, more than 40 different species of micro-algae, isolated in different parts of the world, are cultured as pure strains in intensive systems [32, 33, 34]. It includes species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometers to more than 100 μm. The most frequently used species in commercial mariculture operations are the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, *Monochrysis lutheri* and the chlorococcalean *Chlorella* spp. [30, 31]

ALGAL PRODUCTION

Physical and chemical conditions

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. The most optimal parameters as well as the tolerated ranges are species specific and a broad generalization for the most important parameters. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another. [36, 61, 65]

Culture medium/nutrients

Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate. Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. [37] Micronutrients consist of various trace metals and the vitamins thiamin (B1), cyanocobalamin (B12) and sometimes biotin. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium and the Guillard's F/2 medium.

Commercially available nutrient solutions may reduce preparation labour. The complexity and cost of the above culture media often excludes their use for large-scale culture operations. Alternative enrichment media that are suitable for mass production of micro-algae in large-scale extensive systems contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers. [50, 43, 71]

Light

As with all plants, micro-algae photosynthesize, *i.e.* they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for Erlenmeyer flasks; 5,000-10,000 is required for larger volumes). [38, 67] Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develops normally under constant illumination. [68, 69]

pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth. [77, 78, 70]

Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air.

The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (*e.g.* at a rate of 1% of the volume of air). CO_2 addition furthermore buffers the water against pH changes as a result of the CO_2/HCO_3 - balance. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, Erlenmeyer's), aerating (bags, tanks), or using paddle wheels and jet pumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing. [89, 90, 91]

Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold

water over the surface of the culture vessel or by controlling the air temperature with refrigerated air- conditioning units.

Salinity

Marine phytoplanktons are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 g/l have been found to be optimal. [40, 58, 75]

ISOLATING/OBTAINING AND MAINTAINING OF CULTURES

Sterile cultures of micro-algae used for aquaculture purposes may be obtained from specialized culture collections. Alternatively, the isolation of endemic strains could be considered because of their ability to grow under the local environmental conditions. Isolation of algal species is not simple because of the small cell size and the association with other epiphytic species. Several laboratory techniques are available for isolating individual cells, such as serial dilution culture, successive plating on agar media, and separation using capillary pipettes. Bacteria can be eliminated from the phytoplankton culture by washing or plating in the presence of antibiotics. The sterility of the culture can be checked with a test tube containing sea water with 1 g. 1-1 bactopeptone. After sterilization, a drop of the culture to be tested is added and any residual bacteria will turn the bactopeptone solution turbid. The collection of algal strains should be carefully protected against contamination during handling and poor temperature regulation. To reduce risks, two series of stocks are often retained, one which supplies the starter cultures for the production system and the other which is only subjected to the handling necessary for maintenance. Stock cultures are kept in test tubes at a light intensity of about 1000 lux and a temperature of 16 to 19°C. Constant illumination is suitable for the maintenance of flagellates, but may result in decreased cell size in diatom stock cultures. Stock cultures are maintained for about a month and then transferred to create a new culture line. [54, 55, 56, 57, 58]

ALGAL CULTURE TECHNIQUES

Algae can be produced using a wide variety of methods, ranging from closely-controlled laboratory methods to less predictable methods in outdoor tanks. The terminology used to describe the type of algal culture includes:

• Indoor/Outdoor. Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.

• Open/Closed. Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

• Axenic (=sterile)/Xenic. Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination. The latter makes it impractical for commercial operations.

• Batch, Continuous, and Semi-Continuous. These are the three basic types of phytoplankton culture. [100, 102]

ALGAL PRODUCTION COST

The estimates of the algal production cost range from US $ 4 to 300 per kg dry biomass. Algal production in outdoor ponds is relatively cheap, but is only suitable for a few, fast-growing species and is characterized by a poor batch-to-batch consistency and unpredictable culture crashes due to contaminations and/or fluctuating climatological conditions. Indoor algal production offers a better control of the culture conditions and the algal species being grown, but is more expensive than outdoor culture due to space, energy, and skilled labour requirements. An international survey among the operators of bivalve hatcheries showed that only facilities capable of producing mass quantities of specific micro-algae are able to attain production costs below US $ 100 per kg of dry weight. [49, 67, 78]

NUTRITIONAL VALUE OF MICRO-ALGAE

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 12-35%, 7.2-23%, and 4.6-23%, respectively. The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine organisms. Significant concentrations of EPA are present in the diatom species (Chaetoceros calcitrans, C. gracilis, S. costatum, T. pseudonana) and the prymnesiophyte Platymonas lutheri, whereas high concentrations of DHA are found in the prymnesiophytes (P. lutheri, Isochrysis sp.) and Chroomonas salina. Micro-algae can also be considered as a rich source of ascorbic acid (0.11-1.62% of dry weight). The nutritional value of micro-algae can vary considerably according to the culture conditions. [61, 89, 90, 95]

The protein content per cell, which is considered as one of the most important factors determining the nutritional value of micro-algae as feed in aquaculture, was found to be more susceptible to medium-induced variation than the other cellular constituents. Moreover, the growth of animals fed a mixture of several algal species is often superior to that obtained when feeding only one algal species. [13] A particular alga may lack a nutrient, while another alga may contain that nutrient and lack a different one. In this way, a mixture of both algal species supplies the animals with an adequate amount of both nutrients.

APPLICATIONS OF MICROALGAE AS BIOFUELS

Microalgae are microscopic organisms that typically grow suspended in water and are driven by the same photosynthetic process adopted by higher plants. However, unlike higher plants, algae do not require a vascular system to transport nutrients, because as every cell is photoautotrophic, they can directly absorb the dissolved nutrients. Conventional terrestrial plants are relatively

inefficient in capturing light, converting less than 0.5% of the solar energy received at typical mid latitudes into plant into plant biomass; in contrast, the photosynthetic efficiency of microalgae potentially can exceed 10.0%. [75, 76, 77, 78].

Through the process of photosynthesis, microalgae convert water, carbon dioxide and light into oxygen and biomass. The carbon source that is required for the cultivation of microalgae represents 60.0% of the costs for the nutrient. The use of alternative sources such as CO_2 emitted from the burning of coal in power plants, as well as minimizing the problems caused by the emission of this gas, such as global warming, reduces costs with this nutrient and generates carbon credits that can be traded with countries that need to reduce emissions of greenhouse gases.

The production of 100 tons of microalgal biomass fixes 183 tons of CO_2. (Figure 2) [79] Microalgae can be grown on land unsuitable for agriculture and farming, or on inhospitable land such as deserts, using brackish water and/or wastes from the desalination process that, depending upon their composition, the cultivation medium may be added to the crop limiting nutrients. As fresh water is an increasingly scarce resource, the cultivation of microalgae improving water quality through the economical and environmentally-appropriate use of it.

Microalgae can double their biomass in mean times that range from 2 to 5 days, achieving large yields, without the need for the application of pesticides, herbicides or fungicides. Doubling the biomass of terrestrial plants, genetically modified or not, takes months, and doubling the concentration of animal protein can take years. The production of protein from microalgae consumes three times less water than producing the same amount of protein from soy, which is the plant that is richest in this nutrient. [80, 81].

Energy production from microalgae, when compared to other traditional forms of energy such as wind, hydro or from other biomass such as plants, household and industrial waste, has the advantage of simultaneously fixing large amounts of carbon dioxide. The search for solutions to energy problems requires understanding what the alternatives are and choosing the most appropriate ones. These choices may affect the local patterns of consumption and quality of life of people. The choice of biofuels or raw material used for their production should take into account the conservation of sensitive ecosystems and biodiversity, competition for land with food and settled populations, the preservation of water resources and the impact of fertilizers for pest control. The composition and rates of photosynthesis and growth of these organisms are strongly dependent on growing conditions, so that manipulation of these can result in a greater production of metabolites that are of interest. [82] At the end of the process, according to the characteristics of the microalgal biomass obtained, it can be used to produce biodiesel, ethanol, hydrogen, biogas or direct burning and are not precursors of problems caused by fossil fuels and renewable energies. [83, 84]

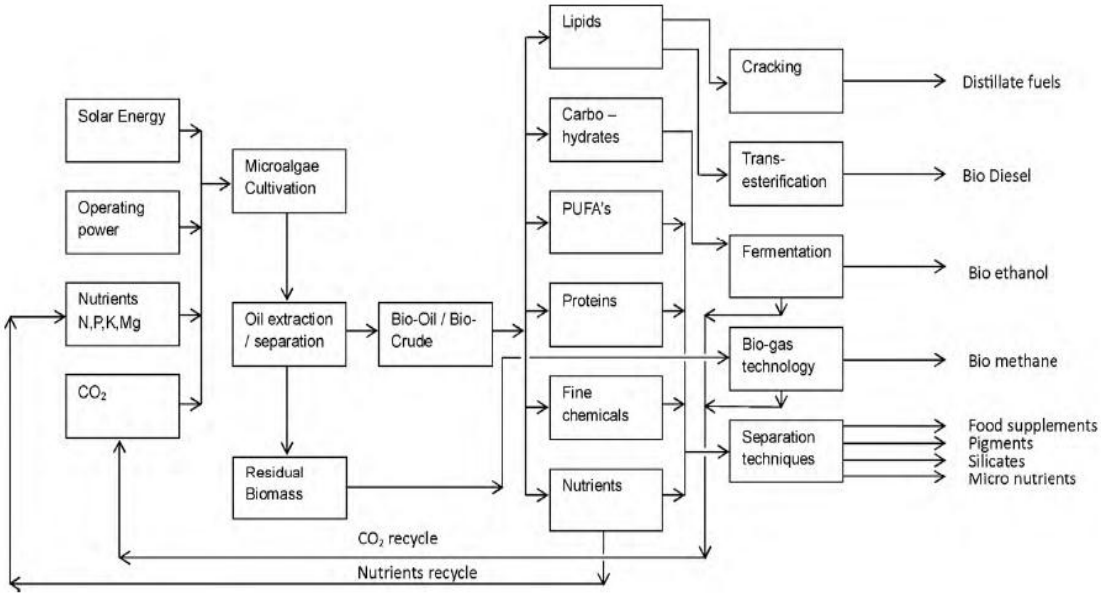

Fig. 2. Proposed schematic flow sheet for a microalgae biorefinery. (79)

USE OF MICROALGAE FOR ASSIMILATION AND UTILIZATION OF CARBON DIOXIDE FROM FOSSIL FUEL-FIRED POWER PLANT FLUE GAS

Carbon dioxide is viewed as a potential agent of global climate change. Anthropogenic emissions of carbon dioxide are estimated to be 2 x 10" tons/y, primarily from combustion of fossil fuels. In addition, the increasing global population pushes the demand for economic energy sources higher every year. Contrasted with its potential for harm is the value of carbon dioxide - it is the source of carbon for photosynthesis, without which there would not be life on earth as we know it today. Carbon dioxide is a valuable resource for many of man's activities including enhanced oil and gas recovery, urea production, and food and beverage carbonation. These activities and others of similar nature,

however, can use only a very small percentage of the total carbon dioxide emissions from fossil fuel combustion. [55, 56]

Using carbon dioxide from fossil fuel combustion as a feedstock for photosynthetic microorganisms can provide a large sink for carbon assimilation. Microalgae are the most productive carbon dioxide users with yields of biomass per acre threefold to fivefold greater than that from typical crop plant acreage. Earlier studies have discussed the potential applications of power plant flue gas to microalgal farming. [85]

HYDROGEN PHOTOSYNTHETICALLY PRODUCED BY MICROALGAE

World consumption of hydrogen is around 1012 m^3 per year. Around 250 - 109 m^3 is used in the production of ammonia and another 250 - 109 m^3 is divided between the petrochemical industry and other applications. The use of hydrogen as a fuel causes less environmental impact, which is why many studies have been carried out on this element, so that it can play a more significant part in the energy consumption of many countries. When burned as a conventional fuel, whether in stationary engines, gas turbines, automotive vehicles, or boilers and heaters, the use of hydrogen leads to the emission of practically just one type of pollutant, NOx. Microalgae have the genetic, metabolic and enzymatic characteristics for photoproduction of hydrogen. Its capacity for synthesis is linked to the exposure of the microalgal cultivation to certain conditions, and closed systems that facilitate the capture of hydrogen gas can even be used. The photobiological production of hydrogen can be increased according to the biomass's carbon content. In the late 19th century it was reported that a natural bloom of the cyanobacterium Anabaena, when placed into a glass jar, rapidly started to evolve hydrogen gas. The first scientific investigation of H_2 evolution by microalgae demonstrated that after a period of dark anaerobic 'adaptation', the green alga Scenedesmus obliquus produces H_2 in the dark at low rates, with H_2 production greatly stimulated in the light, though only for relatively brief periods. [91] Other noteworthy observations were that uncouplers and low CO_2 concentrations stimulated light driven H_2 production in green microalgae. Under anaerobic conditions, the eukaryotic microalgae produce hydrogen as an electron donor in the process of CO_2 fixation. During photosynthesis, the microalgae convert water molecules into hydrogen ions (H^+) and oxygen. The hydrogen ions are then converted into H_2 by the enzyme hydrogenase. The photosynthetic production of O_2 results in rapid inhibition of the enzyme hydrogenase and the production of H_2 are inhibited. Therefore, cultivation of microalgae for the production of hydrogen must take place under anaerobic conditions.

Two methods can be used for the photosynthetic production of hydrogen. In the first method, the hydrogen production takes place in two stages, where the synthesis of hydrogen and oxygen occur partially separated. In the first stage, the algae grow photosynthetically under normal cultivation conditions. During the second stage, the microalgae are exposed to anaerobic conditions and sulphur is limited. With this process system, no toxic products are generated, and compounds with high added value can be produced as a result of the microalgal cultivation. [91, 92]

The second method involves the simultaneous production of oxygen and hydrogen. In this method, the electrons that are released by the photosynthetic oxidation of water are used by the hydrogenase. The production of hydrogen is theoretically superior in the two-stage method, while the simultaneous production is rapidly inhibited by the action of oxygen. The first stage to produce hydrogen by microalgae, the CO_2 fixation into storage carbohydrates would be carried out in large (several hectares) unlined, paddle wheel mixed, raceway-type ponds. Such pond systems are used in both wastewater treatment and for commercial microalgae production. [60] The algal culture would then be concentrated 10- to 20-fold by settling, and held in a large anaerobic fermenter (small, deep, covered lagoons) to induce the hydrogenase enzyme and initiate H_2 production, along with other metabolites, such as acetate. It was assumed that one-third of the H_2 stored in the carbohydrates would be released by anaerobic fermentations. Then the culture would be transferred to closed photobioreactors that expose the cells to light, converting acetate and remaining carbohydrates to H_2. Finally the depleted cells would be recycled to the open ponds to repeat this cycle, for a total of ten times in this conceptual analysis. A small pilot plant was operated in Japan for several years, using two 2 m^2 of open ponds for producing a green algal (Chlamydomonas reinhardtii) biomass and about 2 m^2 of closed photobioreactors, with an intermediate dark fermentation stage. In an impressive demonstration of the largest and longest-duration microalgal biohydrogen production project carried thus far, these researchers successfully operated this process on a continuous basis, producing several litres of H_2 per day. They demonstrated the ability to recycle algal cultures repeatedly through the process, which included a separate dark fermentation stage and a hollow fibre ultrafiltration unit to separate the algal fermentation products and feed these to the photosynthetic bacterial photobioreactors. [93, 59, 87]

MICROALGAE IN COSMETICS

Some microalgal species are established in the skin care market, the main ones being Arthrospira and Chlorella. Some cosmeticians have even invested in their own microalgal production system (LVMH, Paris, France and Daniel Jouvance, Carnac, France).

Microalgae extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers). Microalgae are also represented in sun protection and hair care products. Here are two examples of commercially available products and their properties claimed by their companies; a protein-rich extract from Arthrospira repairs the signs of early skin aging, exerts a tightening effect and prevents stria formation (Protulines, Exsymol S.A.M., Monaco); and an extract from Chlorella vulgaris stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction (Dermochlorella, Codif, St. Malo, France). Recently, two new products have been launched by Pentapharm (Basel, Switzerland): an ingredient from Nannochloropsis oculata with excellent skin-tightening properties (short and long-term effects) (Pepha-Tight) and an ingredient from D. salina, which shows the ability to markedly stimulate cell proliferation and turnover and to positively influence the energy metabolism of skin (Pepha-Ctive). [89, 78]

BIOELIMINATION OF CONTAMINANTS BY USING MICROALGAE

Bioremoval is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological materials, thus allowing the recovery and/or environmentally acceptable disposal of the pollutants. Either plant or microbial biomass can be used for this purpose; the latter is more commonly used. The idea of using microalgae in bioremoval processes has gained interest in recent years. The high concentration of inorganic nitrogen compounds present in streams constitutes a considerable problem for wastewater treatments. This directly affects the quality of the water for domestic and industrial uses. Frequently, primary and secondary treatments are not effective for removing nitrogen and phosphorus compounds; thus, in order to avoid secondary contamination produced by tertiary wastewater treatments, biotechnological processes are more recommended than chemical ones. Microalgae systems are able to efficiently eliminate nitrogen and phosphorus compounds responsible for eutrophyzation problems. [54, 56] The use of microalgae for bioremoval shows several advantages utilization of a cheap and abundant energy source (sunlight); the production of biomass for animal feed; and the production of high added-value compounds and fine chemicals. In contrast, microalgae present problems for removing the biomass from the water course, which may recommend their immobilization or the use of higher plants as alternative for nutrient removal. In addition, the use of bioreactors is widely extended. They are an important tool for applied, processes using microorganisms. Airlift bioreactors (ALR) are a relatively new type of fermenter, offering several advantages for large-scale bioprocesses in which the content is pneumatically stirred by a stream of air or other gases. Furthermore, this stream also has the important function of favouring the exchange between the gas phase and the medium, and the flow will depend on the geometry of the system. Bioreactors have been used with microorganisms in productive processes or in bioremoval processes such as inorganic nitrogen removal or metal biosorption or accumulation. [57]

INORGANIC NITROGEN ELIMINATION

Due to excessive utilization of fertilizers in agriculture and the increasing accumulation of human and animal wastes, inorganic nitrogen concentration has risen in rivers and streams as well as in underground aquifers which precludes their exploitation as potable water. Since immobilization is the most promising technique for new technologies in wastewater treatment, the researchers focused their attention on developing immobilized cell systems to remove nitrogenous compounds from water. [58] Some bacteria including *Nitrosomonas* and *Nitrobacter are* able to oxidize ammonium to nitrate (nitrification) which can be further converted to atmospheric nitrogen by denitrifying bacteria such as *Pseudomonas.* Both, nitrifying and denitrifying bacteria could be used to complete the conversion of ammonium into nitrogen. [101]

On the other hand, the ability of microalgae to assimilate inorganic nitrogen into biomass could be very effective for nitrogen compound detoxification. It has also been studied the nitrite uptake from water as an initial step required to establish optimal conditions for the potential use of the system in bioreactors. A group of parameters such as matrix concentration, cell loading, temperature, or pH has to be considered in order to determine the best working conditions for immobilized cells. In the case of C. *reinhardtii* cells, an alginate concentration of 3% is adequate for minimizing substrate diffusion problems, thereby allowing the attainment of beads with a physical consistency that avoids cell leaking and/or the disruption of the system. Immobilized cells in a discontinuous flow reactor system showed a maximum nitrite uptake rate of 90 µmol h^{-1} at a cell loading of 90 µg Chl g^{-1} gel while the rate was 120 µmol h^{-1} in a continuous flow reactor for 120 µg Chl g^{-1} gel of cell loading [110]. These rates could be maintained for at least 21 days. The stability of the immobilized system (C. *reinhardtii* entrapped in calcium alginate beads) allows the cells to conserve the basic biological activities (photosynthesis and respiration) during at least one month by operating in a continuous mode under cell washout conditions and maintaining a constant nitrite uptake rate. [12]

Particularly interesting from a biotechnological point of view are the specific advantages of immobilized C. *Reinhardtii* cell systems compared to the freely suspended ones: a) cells show high resistance to nitrite toxicity; b) the ammonium-dependent inhibition of nitrite assimilation by C. *reinhardtii* is minimized; and c) the high stability of the system (mainly concerning the cells viability). [71, 72, 73, 74]

METAL REMOVAL FROM WASTEWATERS

Biological systems to remove metal ions from polluted waters could emerge as the potential alternative to chemical treatments. Nowadays, the process includes the addition of chemicals for metal precipitation or exchange resins to bind them. Other less frequently employed methods are activated carbon adsorption, electrodialysis, and reverse osmosis. One of the main interests for microalgae in biotechnology is focused on their use for heavy metals and radionuclide removal from effluents and wastewaters. In parallel to detoxification, it is also possible to recover valuable elements such as gold and silver after appropriate treatment of the loaded microbial biomass. The metal bioremoval process mainly combines two types of mechanism. [75]

Passive uptake (metabolism-independent)

It is called *biosorption*, and it occurs when the metal ion binds to the cell wall according two different ways: a) ion exchange where monovalent and divalent ions in the cell wall are displaced by heavy metal ions; and b) the complex formation between metal ions and functional groups present at the cell wall. Biosorption is reversible and rapid (it is completed in 5-10 min). The amount of metal accumulated per unit of biomass is proportional to the concentration of metal ion in the solution.118 In addition, biosorption can be affected by pH and the presence of other ions in the medium which may precipitate heavy metals as insoluble salts, but it is unaffected by metabolic inhibitors, uncouplers, or light/dark cycles. [76]

Active uptake (metabolism-dependent)

This mechanism may simultaneously involve metal ion consumption for the microalgae growth and/or intracellular accumulation of metal ions. In addition, heavy metals could also be precipitated by excreted secondary metabolites. These processes are energy dependent and sensitive to different parameters including pH, temperature, ionic strength, light, etc. They are inhibited by low temperature, absence of energy source, metabolic inhibitors, and

uncouplers. Active uptake is more effective than biosorption for low metal ion concentrations. Below 1 ppm the cleaning could be accomplished without excessive utilization of biomass. [81, 92] Both mechanisms can work simultaneously in microalgae, and their relative importance may depend on the algal specie, culture conditions, and the metal chemical properties. In *Ankistrodesmus braunii* and *Chlorella vulgaris,* cadmium binding to cell walls accounted for approximately 80% of total uptake. . Biosorption was the major uptake component in C. *vulgaris* with respect to a wide range of other metals including uranium. In *Eremosphaera viridis* however, intracellular uptake comprised the majority of total uptake. A wide range of metal ions is bounded by living cells of microalgae: Cu^{2+}, Zn^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Sr^{2+}, Hg^{2+}, and Ag^{1+} as principals. [93]

Proteins and polysaccharides play significant roles in metal biosorption while covalent bonding likely involves amino and carbonyl groups. Sometimes, dead biomass of microalgae is directly used for metal biosorption, working the cell wall like a metal ion exchanger. Biosorption of heavy metals and radionuclides by a variety of freshwater and marine algae shows a linear equilibrium relationship between the metal concentration in solution and that bound to the cell surface. Cadmium adsorption by nonliving biomass of microalgae and the use of algal biomass for mercury extraction from groundwater have been reported. The alga was immobilized in a permeable polymeric matrix. The product (AlgaSORB, packed into adsorption columns) exhibited excellent flow characteristics and acted like a "biological" ion-exchange resin. Mercury also accumulated in immobilized *Chlorella emersonii* cells. They removed 99% after 12 days (initial concentration of mercury, 1 mg l^{-1}) with an initial cell loading of $l0^6$ cells $bead^{-1}$. [94, 95]

Chlorella homosphaera cells immobilized in alginate supply a good system to remove cadmium, zinc, and gold from water. When the initial concentration of heavy metals ranged between 20-720 mgl^{-1}, 99% of Cd and Zn were removed after 60 min and 90% after 30 min (40% associated with matrix). Cadmium removal was also reported by Volesky and Prasetyo using a new biosorbent material derived from a brown marine alga *(Ascophyllum nodosum)* in a packed-bed flow-through column. It reached a 99.98% removal from an effluent containing 10 mg Cd l^{-1}. *Chlorella salina* cells accumulated Co, Zn, and Mn when they were immobilized in alginate (0.95; 1.12; 0.74µg 10^{-6} cells after 5 h). Avery *et al.* reported that these cells may remove 70% of Cs from liquid medium after 15 h of continuous process in which the initial metal concentration was 6.5 mg l^{-1}

Screening tests of different marine algae biomass types revealed a high passive biosorptive lead uptake up to 270 mg Pb g^{-1} biomass. This limit was increased to 370 mg Pb g^{-1} in cross-linked *Fucus vesiculosus* and *Ascophyllum nodown;* however, ion-exchange resin Amberlite IR- 120 presents a higher lead uptake than biosorbent materials. In all cases, an order of magnitude lower uptake of nickel was observed. Accumulation of lead and zinc from metalliferous spoil tip stream has been seen by using different algae. Freshwater *Chlorella, Scenedesmus,* and *Chlamydomonas* sp. were capable of taking up significant amounts of uranium. Green algae and cyanobacteria were found to have fewer reactive surfaces than diatoms. Particularly interesting is the heavy metal tolerance in microalgae. [79, 80, 96, 97] Certain specific peptides and proteins binding metals could become over expressed in microorganisms when growing in the presence of heavy metals. The identification of the genes responsible for this resistance mechanism would be of biotechnological interest to improve the metal accumulation capacity of different microorganisms. It is well known about the capacity of *Chlamydomonas* to grow in the presence of metal ions or even to accumulate them. C. *Reinhardtii* can grow with strontium replacing calcium in the culture medium. Metal-resistant strains of *Dunaliella tertiolecta* and *Scenedesmus acutus* have been isolated, but their resistance was gradually lost when the cells were grown in the absence of metal. This suggests that, in both cases, the tolerance results from physiological adaptation. This has been described for C. *vulgaris, Euglena gracilis, Stigeoclonium tenue,* C. *reinhardtii* in the presence of heavy metals. By growing C. *reinhardtii* cells into an agar medium containing cadmium at a concentration that induces high lethality, stable resistant clones were isolated which apparently contain a nuclear mutation. [98, 99]

In addition, mutants resistant to cadmium, copper, zinc, cobalt, and nickel have been isolated. It has been investigated that the manganese uptake is by *Chlamydomonas* sp. Zinc uptake has been studied in C. *Variablli.* The ability of *Chlamydomonas* to accumulate heavy metals can be used to design effective cell systems for metal ion removal. The identification of the mechanism involved (specific peptides or proteins, lipids, etc.) in metal ion intracellular accumulation should allow for design of the optimal conditions for efficient systems and the attainment of mutants capable of living under high metal ion concentrations. [45, 46, 50] The use of immobilized cells probably should increase the yield of the metal removal processes due to the capacity of the matrix to behave as an ionic exchanger. When metal recovery is of economic interest, the technological method used will depend on the mechanism of metal accumulation by microalgae. It is possible to use a non-destructive method which requires regeneration of biomass for reuse. Destructive recovery may be accomplished by treatment of biomass with strong acids or alkalis. Most attention has been focused toward non-destructive desorption from loaded biomass. [100, 101, 111]

POTENTIAL PRODUCTS AND GENETIC ENGINEERING

One use of the biomass produced by microalgal mass culture using the carbon dioxide from flue gas is co-combustion for energy generation. Although CO would still be released to the atmosphere, there would be an overall reduction in the amount per unit of energy produced as the microalgae would essentially recycle the carbon from the power plant stack. [51, 52, 53] Alternatively, the biomass can be used to produce transportation fuels (such as biodiesel) or commodity chemicals, again acting to decrease the carbon dioxide generated by the use of petroleum products. All of the suggested scenarios would serve to lower the nation's dependence on imported oil. [54, 55]

Recent research efforts have concentrated on applying genetic methods to microalgae in order to develop organisms optimized for high productivity and energy value. DNA from microalgae has been analyzed chemically, and transient expression of a foreign gene (firefly luciferase) was demonstrated. [68, 64, 85] Progress has also been made with

respect to the development of new protocols for introducing foreign genes into microalgae. In the area of microalgal lipid biochemistry and molecular biology (important for production of biodiesel), an important enzyme in lipid metabolism, acetyl-CoA carboxylase was purified. This purified enzyme aided efforts to clone and sequence this key gene in lipid metabolism. Genetic engineering studies should result in organisms with desired traits, thereby improving process feasibility. [87, 88, 89, 90, 91]

Combining microalgal farming with fossil fuel energy production has great potential to diminish overloading of the atmosphere with carbon dioxide, as well as for production of useful products (i.e., energy from combustion of biomass, transportation fuels such as biodiesel or commodity chemicals) [112].

The laboratory studies described here demonstrate that microalgae can efficiently utilize simulated flue gas containing high levels of carbon dioxide, as well as sulphur and nitrogen oxides, as a feedstock to produce substantial biomass. Several parameters important for productivity have been identified including nutrient levels and sparging regime. Operation of the mass culture facility in New Mexico demonstrated that there are no significant engineering barriers to large scale pond culture. The implementation of this technology is eminently feasible and that while further investigation into culture physiology and other areas will refine process design, no major technological breakthroughs are needed. [39, 40]

CONCLUSIONS

A critical assessment of the commercialization potential of algae biofuels has been presented. The suitability of algae feedstocks for conversion to biofuels has been explored. It seems that new technologies, e.g. tubular PBRs shall enhance the production of microalgae feedstocks for various fuel productions along with CO_2 recycle for algae culture and thus reduce the pollution. Some life cycle analyses have been presented by few authors. A critical analysis of these studies reveals that an adequate LCA study is still not available which may help to present a clear picture of the situation. The reason is non-availability of commercial plant data. It may be hoped that with more and more companies coming forward in microalgae conversion business, a more detailed picture will emerge out. Since the microalgae feedstocks are noncompeting with land use change as well as food crops, the scope of their implementation looks great. It is obvious from the critical appraisal of the viability of algae projects from a true market perspective that total fixed costs along with recurring costs will be a decision-making step to the future commercialization of the algae-based biofuels. More innovations are still needed for the development of technologies which reduce costs while increasing the yields. This can be realized successfully through a coherent, extensive, and well-funded R&D program. It is extremely important in the early phases of this promising, yet challenging industry, to deliberate new business models that look at the bioenergy potential of algae through the transportation fuels market, as well as production of other higher value products so as to make the economics practicable. A sustained effort from the technologists and planners can result in the successful accomplishment of this extremely potential concept towards the solution of world's future energy concerns. [95, 96, 97]

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